PB86-100179

Determination of Fenthion Residues in Samples of Marine Biota and Seawater from Laboratory Exposures and Field Applications

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1985

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1. REPORT NO.	2.	3. RE
EPA/600/J-85/124		PB86-100 179

4. TITLE AND SUBTITLE	5. REPORT DATE
DETERMINATION OF FENTHION RESIDUES IN SAMPLES OF MARINE BIOTA AND SEAWWATER FROM LABORATORY EXPOSURES AND FIELD APPLICATIONS (Journal Version)	1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
E.M. Lores, J.C. Moore, J. Knight, J. Forester, J. Clark, and P. Moody	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
same as #12	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory - Gulf Breeze, FL Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561	14. SPONSORING AGENCY CODE EPA/600/4

15. SUPPLEMENTARY NOTES

J. Chromotogr. Sci. 23:124-127 March 1985

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	5
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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EPA/600/J-85/124
JOURNAL ARTICLE

Determination of Fenthion Residues in Samples of Marine Biota and Seawater from Laboratory Exposures and Field Applications

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Abstract

A method for measuring fenthion in samples from the estuarine environment is described. The method was applied to samples from the field applications of fenthion to control salt-marsh mosquitos. The method offers an improvement in the silica gel cleanup of Thompson et al.(1) and yields >85% recovery of fenthion from water, plant, fish, and shrimp tissues. Gas-liquid chromatography with thermionic detection was used to quantify fenthion residues as low as 0.010 μg/l in seawater and 0.010 μg/g in biota. Concentration of fenthion residues ranged from undetectable to 0.68 μg/l in saltwater samples collected after truck-mounted ultra-low-volume or aerial spraying to control salt-marsh mosquitos.

Introduction

Fenthion, *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate, an organophosphate insecticide used extensively in the state of Florida, was selected for a field study to examine the applicability of laboratory toxicity data in predicting environmental effects of a pesticide under actual-use conditions. During fiscal year 1980, 34,639 liters of this insecticide (93% active ingredient) were used in Florida for state-supported mosquito control (2). Before the field study could begin, methods of extraction and quantitation had to be validated for samples of marine biota and sediments.

Several techniques for extraction and quantitation of fenthion residues were found in the literature. The methods for quantitation of fenthion in freshwater (3-6) did not require cleanup and, in general, detection limits were not low enough for the authors' purposes. Anderson et al. (7) used a hydrolysis and bromination of the phenol, a method that was too lengthy for the present purposes, considering the number of samples to be analyzed. Aoki et al. (8) used reagents that are not readily available in this country. The method of Ferreira and Fernandes (9) for extraction of pesticides from fruits and vegetables used florisil, which did not work for cleanup of water and tissue extracts in the authors' laboratory because, in general, the recovery was < 25% from florisil cleanup columns. The remaining methods (10-12) all used benzene, which is avoided in the authors' laboratory since it was found to be a carcinogen.

Quantitative extraction and cleanup methodology had to be developed to measure fenthion in estuarine water and biota at the low concentrations expected following field application (0.010 to 100 μg/l in seawater, 0.0010 to 10 μg/g in biota).

Experimental

The field sites selected for testing were two lagoons opening directly into Santa Rosa Sound in a salt-marsh area on Santa Rosa Island near Fort Walton Beach, Florida. Truck-mounted ultra-low-volume (ULV) sprayings were conducted at these sites. Site 1 (ca. 7 m from the road) had a maximum depth of 1.3 m; Site 2 (50 m from the road) was shallower with a maximum depth of 0.3 m. Sampling stations were selected within each lagoon. In a second study, conducted in southern Florida, routine aerial application of fenthion was monitored by analyzing oysters and water samples.

Apparatus

A Model 5710 gas-liquid chromatograph (GLC) (Hewlett-Packard) equipped with nitrogen-phosphorus detectors and 182-cm × 2-mm i.d. glass columns packed with either 5% QF-1 on 80/100 mesh Gas Chrom Q or 0.75% OV-17/0.97% was OV-210 on 100/120 mesh Supelcoport mixed-phase packing was used for analyses. Operating conditions were: oven temperature, 200°C; injector temperature, 200°C; detector, 300°C; helium carrier gas flow, 30 ml/ min. Detector flow rates were 4.0 ml/min hydrogen and 100 ml/min air.

Reagents

All solvents were nanograde (Burdick and Jackson Laboratories) or equivalent. Silica gel (E. Merck) was "Silica Gel 60," particle size 0.063 to 0.200 mm. Reagent grade glacial acetic acid (Baker) was used to make the 1% (v/v) acetic acid in hexane for silica gel column conditioning and elution.

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Standards

A fenthion standard (Lot C-102, 97.2% pure) was obtained from the U.S. Environmental Protection Agency (EPA) Pesticide Repository. The primary standard (1.0 mg/ml) was prepared in *p*-xylene; working standards (0.040 ng/μl) in hexane. Sediment and biota were spiked with fenthion dissolved in acetone; however, hexane solutions of fenthion were used in determining recoveries from silica gel columns.

Procedures

One-liter water samples were collected in 1.0-liter borosilicate glass bottles (Wheaton); after 100 ml of petroleum ether were added, the bottles were stored in styrofoam containers packed with ice and transported to the laboratory for extraction and quantitation. Samples were usually analyzed within 24 hr after being received; however, unrefrigerated water samples fortified with 1.0 μg of fenthion/l were stored for four days without measurable loss of fenthion residues. Seawater samples were extracted twice with 100-ml aliquots of petroleum ether by shaking for 1 min in a 2-l separatory funnel. The solvent was dried by passing it through a funnel containing heat-treated (600°C) glass wool and was collected in a Kuderna-Danish 500-ml evaporator fitted with a Snyder three-ball column and 25-ml concentrator tube. The solvent was evaporated on a steam cabinet to 10 ml, then concentrated to 1 ml with a gentle stream of nitrogen at 35°C.

Tissue and sediments were stored on ice, transported to the laboratory, and frozen until analyzed (usually within seven days). These samples were prepared for analysis by placing from 1 to 8 g of tissue in a 150-mm × 25-mm screwcap culture tube and extracting with three 10-ml volumes of acetonitrile by homogenizing with a Willems Model 10-ST Polytron (Brinkman Instruments). Following each homogenization, the test tube was centrifuged (1600 × g) and the extract decanted into a 120-ml oil sample bottle (American Scientific Products, Cat. #B7915-2) where 75 ml of 2% (w/v) aqueous sodium sulfate and 10 ml of petroleum ether were added. The contents were shaken for 1 min and the layers allowed to separate. The petroleum ether extract was transferred by pipet into a 25-ml concentrator tube and the contents extracted two more times. The combined solvent extract was concentrated to 1 ml with a gentle stream of nitrogen at 35°C.

One to three grams of *Juncus* grass (*Juncus roemerianus*) were weighed into a 150-mm × 25-mm screwcap test tube and extracted with the same procedure used for tissue, except that the homogenization and centrifugation were repeated a total of five times. The extracts were decanted into a 250-ml separatory funnel with 150 ml of 2% (w/v) aqueous sodium sulfate and 25 ml of petroleum ether, and the biphasic mixture shaken for 1 min. After separation, the petroleum ether extract was concentrated as in tissue preparation.

Surface samplers were designed to determine the amount of fenthion that was deposited on the surface of water during the field sprays. These samplers were made of acrylic plastic and styrofoam and contained filter paper that could be removed for subsequent analysis of fenthion residues. During the first three sprays, 24.0-cm diameter filter paper (Whatman No. 3) was used in the samplers. Number 44 filter paper (Whatman) was used during later sprays because the authors determined it required less cleanup before use. The samplers were constructed so that only 269 cm^2 of filter paper were exposed, and this value was used in all calculations. The samplers were floated on the surface of the water prior to spraying and collected 15 to 30 min after spraying. The filter paper was removed from the samplers and placed in Teflon-lined screwcap glass bottles; 50 ml of petroleum ether were added and the contents shaken for 30 sec before the samples were transported to the laboratory. Two additional 50-ml extractions were completed within 24 hr after samples reached the laboratory; residues were determined and expressed as ng/cm^2.

Silica gel was stored at 130°C, deactivated by addition of 20% (w/w) distilled water, and tumbled on a roto-rack for 2 hr prior to use. Cleanup columns were prepared by adding 3.5 g deactivated silica gel, followed by 2.0 g of anhydrous sodium sulfate (granular), to a 200-mm × 9-mm i.d. Chromaflex® column (Kontes Glass Co.) plugged with glass wool. The columns were washed with 10 ml of 1% (v/v) acetic acid in hexane. Each sample concentrate was transferred to a column using two 1-ml volumes of 1% (v/v) acetic acid in hexane. The column was eluted with 10 ml of 1% (v/v) acetic acid in hexane and the eluant discarded. Fenthion was eluted with 20 ml of 5% (v/v) ethyl ether in hexane. The eluant containing fenthion was concentrated to not less than 1.0 ml with a gentle stream of nitrogen. This concentrate was then injected into a gas chromatograph for quantitation. Data were collected and quaiitatively and quantitatively analyzed on a Model 3357 lab-automation computer system (Hewlett-Packard).

Results and Discussion

The method described is sensitive and efficient for extraction and cleanup of fenthion residues in seawater, marine biota, and sediments. The method detection limits for fenthion in seawater and biota are 0.010 μg/l and 0.010 μg/g, respectively. Detection limits, which are also the authors' limits of quantitation, are based on a signal-to-noise ratio of 10. Average recoveries from seawater, *Juncus* grass, and several types of marine biota fortified with fenthion are > 85%. This procedure has been applied to field samples as well as samples from laboratory toxicity tests.

During the development of the cleanup method, several alternative procedures were investigated. Initial efforts involved use of florisil, but recoveries were extremely low (0 to 25%). Use of water-deactivated florisil improved recovery [20 to 30% for 5% (w/w) deactivation], but silica gel deactivated with 20% (w/w) water gave higher recoveries (range 40 to 60%). At this point, a preliminary test was initiated using a 1% acetic acid in hexane prewash on florisil, silica gel, and alumina columns. The 1% acetic acid in hexane was chosen for evaluation because: (A) most organophosphate pesticides are stable in dilute acid solutions from pH 1 to 5 (13); and (B) acetic acid hexane mixtures have been used for thin layer and paper chromatography for many years. In this preliminary test, recoveries from silica gel and alumina columns using a 1% (v/v) acetic acid in hexane prewash averaged 100%. The 1% acetic acid prewash improved recovery of fenthion from florisil to 80%. The florisil cleanup procedure may be useful for other substrates or when florisil is required for multi-residue application. Silica gel was chosen for use in the authors' method because it gave slightly higher percentage recovery of fenthion, and other pesticide residues were not being quantitated.

Various quantities of silica gel and a number of different elution systems were investigated. In the system chosen, fenthion eluted in the 5% (v/v) ethyl ether in hexane fraction even when the second 10-ml 1% acetic acid in hexane wash was omitted;

however, washing the column with acetic acid/hexane solution should allow possible non-polar contaminants to be removed from fenthion residues. This same elution may also be used with 2.4 g of silica gel, but 3.5 g (5 ml) should provide increased capacity for co-extractives. Recoveries from various substrates spiked with fenthion are shown in Table I. GLC chromatograms of fenthion recovered from fish before and after cleanup are shown in Figure 1. The baseline is much more elevated before cleanup, causing problems with repeated injections and quantitation of small peaks.

Extraction efficiency in whole-body analysis of fish exposed in the laboratory to 20 μg fenthion/l of seawater was determined by extracting tissue homogenates three times with acetonitrile, then once with acetone, and once more with acetonitrile. These tissues contained more than 20 μg of fenthion/g but < 1% was recovered in the additional extracts. Recovery from all substrates was > 85% at all fortification levels, including the method detection limits (0.010 μg/l and 0.010 μg/g).

More than 350 water samples were taken from spray sites after application of fenthion at the recommended rate. Residues of fenthion ranged from undetectable to 0.48 μg/l for the ULV sprays and up to 45 μg/l for the direct application using the recommended rate for freshwater larvicide. Concentrations of fenthion residues in water from ULV and aerial sprays did not exceed 1.0 μg/l even though the solubility of fenthion in seawater was 6.0 mg/l as determined by the method of Schimmel et al. (14).

Tables II and III show the tissue concentrations of fenthion from laboratory- and field-exposed biota. Fish and shrimp used in laboratory tests contained detectable residues of fenthion after 48-hr exposure to 2 μg/l. Laboratory-exposed shrimp died after 12 hr, but still accumulated detectable residues. Field-exposed animals were caged in mesh containers that floated on the surface and extended to a depth of 0.3 to 0.5 m. Samples were collected at 12, 24, and 48 hr post-spray. No field-exposed fish or shrimp accumulated detectable concentrations of fenthion. However, following the direct application of fenthion, a few fish contained traces of fenthion that were below this study's limits of quantitation. Three *Juncus* grass samples nearest the sprayer contained detectable residues. No residues were detected in sediment samples. Oysters taken from the southern Florida aerial spray site had fenthion residues, but oysters were not exposed in the laboratory for comparison.

Surface samplers from the ULV sprays contained residues as high as 6.0 ng/cm². Samplers from the aerial spray contained residues that ranged from 9.5 to 240 ng/cm².

Table I. Percentage Recovery of Fenthion from Fortified Seawater, Fish, and *Juncus* Grass

Fortification*	Percentage recovery								
	Seawater			*Juncus* grass			Fish tissue		
	$\bar{x}$**	SD***	N†	$\bar{x}$	SD	N	$\bar{x}$	SD	N
0.010	85	2.7	5	—	—	—	—	—	—
0.10	101	11.5	9	—	—	—	—	—	—
0.21	104	11.5	6	—	—	—	—	—	—
0.32	100	0	2	—	—	—	—	—	—
0.50	94	2.8	2	90	12.5	4	98	10.5	6
1.0	95	6.6	8	85	8.5	4	100	7.0	4
1.4	93	—	1	—	—	—	—	—	—
2.5	—	—	—	87	7.4	5	88	6.7	3
5.0	—	—	—	85	1.4	2	104	6.2	4
2320	102	0	1	—	—	—	—	—	—

*For seawater, μg/l; for *Juncus* grass and fish tissue, μg/g.
**$\bar{x}$ = sample mean.
***SD = standard deviation.
†N = number of samples.

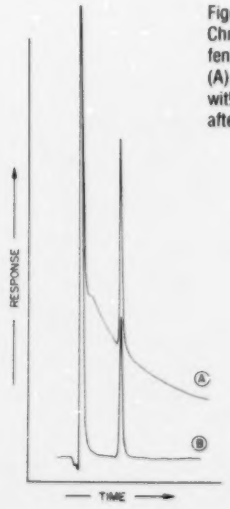

Figure 1.
Chromatograms of fenthion in fish tissue: (A) before cleanup with silica gel; (B) after cleanup.

Conclusion

The silica gel cleanup method was developed for analysis of samples from studies in the estuarine environment. The cleanup method has been successfully tested on a variety of substrates. It required relatively little time, eliminates co-extractives, and

Table II. Fenthion Residues (ng/g) in Tissues of Fishes (*Cyprinodon variegatus, Leiostomus xanthurus, Lagodon rhomboides*) and Pink Shrimp (*Penaeus duorarum*) Exposed in the Laboratory to 2 μg/l for 48 hr

	C. variegatus	*L. xanthurus*	*L. rhomboides*	*P. duorarum**
N**	3	2	6	2
Average	388	1154	72	29
Range	153-559	821-1486	41-136	24-33

*Died after 12 hr and were removed.
**N = number of samples.

Table III. Fenthion Residues (ng/g) in Sediment, Estuarine Biota Including Eastern Oyster (*Crsssostrea virginica*), and *Juncus* Grass (*Juncus roemerianus*) from Field Exposures

	Sediment	*J. roemerianus*	*C. variegatus*	*P. duorarum*	*C. virginica*
N	4	18	12	2	2
Average	ND*	7.2	ND	ND	18
Range	ND	ND-50	ND	ND	17-19

*ND = 0.010 μg/g.

provides adequate sensitivity. Average recovery from tissue is 95%, much better than with previous methods. Work is continuing on the extension of this method to several other organophosphorus pesticides; preliminary tests are promising.

Acknowledgment

The authors thank chemist Miriam Woods for help in analysis of water samples, research biologist D. Middaugh for editing the manuscript, biologists J. Patrick, P. Borthwick, R. Cripe, M. Hemmer, and R. Stanley for collecting samples, Steven Foss and Lee Newkirk for tables and illustrations, and Maureen Stubbs, typist.

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Manuscript received June 22, 1984;
revision received September 27, 1984.